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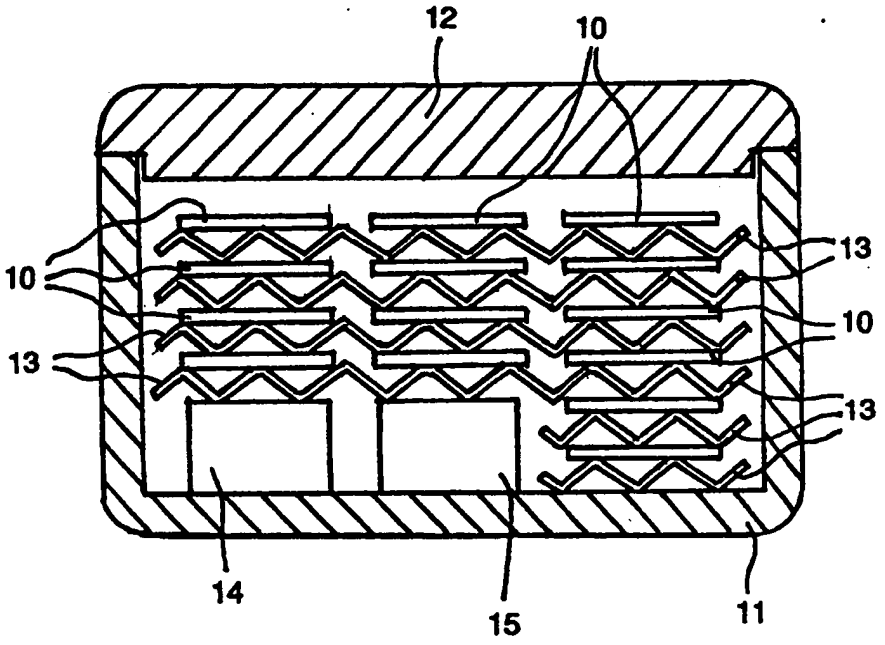
(54) Title: METHOD FOR PACKAGING ENTOMOPATHOGENIC NEMATODES FOR STORAGE AND TRANSPORT

(57) Abstract

To improve the long-term storage of third stage juvenile (J3) entomopathogenic nematodes, the J3 nematodes are combined with a carrier medium so that the combination has a water activity of from 0.85 to 0.99. The combination is formed into at least one package (10), comprising a quantity of the combination wrapped in a polymer film having a permeability to oxygen. Normally, anhydrobiosis of the J3 nematodes is induced before the combination is wrapped in a polymer film. For transporation, the packages (10) are supported within a container (11) on supports (13) which permit air in the container to contact the exposed surfaces of the packages. The relative humidity within the container (11) is maintained at a value of from 85 to 100 per cent, preferabaly using a humidifying pack (15).



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TITLE: "METHOD FOR PACKAGING ENTOMOPATHOGENIC NEMATODES FOR STORAGE AND TRANSPORT"

Technical Field

This invention concerns entomopathogenic nematodes. More particularly, it concerns the packaging, for transportation and storage, of samples of infective third stage juveniles (J3) of entomopathogenic nematodes.

Background

In the last decade, the use of entomopathogenic nematodes for the control of insect pests has been steadily increasing. With the development of techniques for the production of large quantities of infective third stage juveniles (J3) of a range of nematode species and strains, a number of production centres are rearing these nematodes on a commercial scale and sending them by air freight to destinations throughout the world. In most instances, the recipients of the J3 entomopathogenic nematodes do not use them all immediately. Regular use of smaller quantities of nematodes means that some of the received nematodes may be stored for periods of at least one month. In many instances, a longer period of storage of the J3 nematodes is desirable but, using conventional storage techniques, that is not possible.

When transporting and storing entomopathogenic nematodes using conventional techniques, problems arise unless care is taken to ensure that adequate oxygen is available for the nematodes and an appropriate water activity is maintained while they are transported and stored. Storage under refrigeration is also most desirable. Although inactive nematodes have only a small oxygen intake, lack of

that oxygen results in the death of the nematodes (and also the possible release of putricine and cadaverine from the dead nematodes, which would kill neighbouring nematodes). If the water activity of the nematodes in their carrier medium falls to a level which is too low (for example, to a value of 0.8), some of the nematodes will die and others, although alive, will burst when being re-suspended in water (and thus die) or will not regain their original activity level.

One of the currently used methods of storing third stage juvenile entomopathogenic nematodes is described in the specification of International patent application No PCT/AU88/00127, which is WIPO publication No WO 88/08668. That storage technique requires the mixing of the J3 nematodes with a clay (preferably attapulgite clay) while maintaining a predetermined water activity. A water activity as near to 0.97 as possible is suggested, although that publication does imply that lower water activities can be used. The mixture of clay and nematodes is then stored in a selected container. If the storage is not under refrigeration, "some provision [is] made for gaseous exchange between the interior and the exterior" of the container "while minimising water loss". The preferred container of that technique is a rigid plastic container with a series of perforations or holes, about 2 mm in diameter, adjacent to the upper rim of the container.

In general, that method of storage has proved very successful, and suitable for transportation of nematodes from the production centre to the user establishment.

However, as noted above, in the absence of refrigeration, adequate oxygen and control of the water activity in the storage environment, reliable storage of more than one month cannot be guaranteed.

A secondary factor in the storage of nematodes using the attapulgite clay technique referred to above (and other current storage techniques) is that fungal spores can, and do, enter the storage containers and adversely affect the stored nematodes.

Clearly, better utilisation of J3 nematodes for biological control of insect pests could be achieved if the nematodes could be packed for transportation and prolonged storage (up to six months and possibly for a year or more) without the risk of damage due to insufficient oxygen or a reduction of the water activity in the storage environment.

Disclosure of the Present Invention

An object of the present invention is the provision of a method by which third stage juvenile (J3) entomopathogenic nematodes can be packaged for transportation and storage for prolonged periods at a temperature of about 23°C without the need for constant or periodic attention during storage, and without the attendant risk of damage of the stored nematodes due to oxygen deficiency or a low water activity.

This objective is achieved by packaging the nematodes and a suitable carrier material, after anhydrobiosis of the nematodes has been induced (to reduce the rate of oxygen consumption by the nematodes), within an envelope or

wrapper of a polymer material, the polymer material being permeable to oxygen and preferably impermeable to water vapour. For transportation, the packages containing the nematodes and their carrier are then placed within a container, where they are supported on a medium which permits air to be in contact with a substantial portion of the polymer wrappers. Preferably, partially hydrated polyacrylamide gel particles, or particles of another highly absorbent material which has taken up a quantity of water, are also placed within the container, or within at least some of the individual packages, to maintain the relative humidity of the air around the thin packages in the range of from 85 per cent to 100 per cent.

To induce anhydrobiosis, the water activity of the J3 nematodes and their carrier (preferably partially hydrated polyacrylamide gel particles, or the attapulgite clay mentioned above) is reduced, preferably to a value of about 0.97. At this water activity, which corresponds to an external relative humidity of 97 per cent, anhydrobiosis of the nematodes begins and, after a period of from 15 to 48 hours, the nematodes undergo chemical changes which reduce both their activity and their oxygen consumption rate, without affecting their efficacy as an entomopathogenic agent when re-suspended in water. However, as noted above, if, during storage, the water activity of the nematodes and their supporting medium drops below 0.80, the nematodes are likely to die. Consequently, the storage of the nematodes after they have been wrapped by the method of the present invention requires the maintenance of a relative humidity in the storage environment of between 85 per cent and 100

per cent, preferably between 95 per cent and 99 per cent, and most preferably at a value which ensures that the water activity of the nematodes and their carrier medium is optimal for the nematode species that is being transported and stored, which in the case of Steinernema carpocapsae is a water activity of 0.965. For a number of useful nematode species, this most preferred environment requires the maintenance of a relative humidity of about 97 per cent.

Thus, according to the present invention, there is provided a method of packaging infective third stage juvenile entomopathogenic nematodes for transportation and/or storage, said method comprising the steps of:

(a) combining an aqueous cream of the nematodes and a carrier medium in proportions which provide a water activity for the combination in the range of from 0.85 to 0.99;

(b) exposing the combination obtained by step (a) to air for a period sufficient to induce anhydrobiosis of the nematodes; and

(c) forming at least one package of the combination of nematodes and carrier medium, by surrounding a quantity of the combination of nematodes and carrier medium with a film of a polymer material having a permeability to oxygen.

If the package (or packages) of J3 nematodes and their carrier medium are to be transported, then the following additional steps will be undertaken:-

(1) supporting the package (or packages) within a container on a layer (or layers) of a supporting

material which allows air to contact a substantial proportion of the outer surface area of the package (or packages); and

(2) maintaining the relative humidity within said container in the range of from 85 per cent to 100 per cent.

If the polymer material used to wrap the combination of nematodes and carrier medium has a relatively low permeability to oxygen, the packages containing the combination will be thin packages. Thin packages, having a thickness of from 5 mm upwards, ensure that adequate oxygen will reach all of the nematodes in the package. As the permeability to oxygen of the polymer film increases, the thickness of the package may be increased and the shape of the package may be varied. Thus, when the combination of nematodes and their carrier medium is wrapped in a polymer film having a relatively high permeability to oxygen, the package can conveniently be sausage shaped.

If a polymer film having a very high permeability to oxygen is used for the present invention, so that there is always an adequate supply of oxygen to the combination of J3 nematodes and their carrier material, the step of inducing anhydrobiosis can be omitted, and/or the packaging step may become placing a sample of the combination of nematodes and their carrier medium in an open-top box of a plastic material (preferably a shallow plastic box), which is then closed by a membrane comprising the polymer film of very high oxygen permeability.

The permeability of the polymer film used for the envelopes, or for packaging the nematodes, depends upon the

thickness and composition of the film, but for a polymer film having a thickness of 50μ, the permeability to oxygen, carbon dioxide and water vapour should be as follows:

oxygen:-	a permeability of greater than 6×10^{-16} mole.$m^{-1}s^{-1}pa^{-1}$.
carbon dioxide:-	a permeability of greater than 20×10^{-16} mole.$m^{-1}s^{-1}pa^{-1}$.
water vapour:-	a permeability of less than 1000×10^{-16} mole.$m^{-1}s^{-1}pa^{-1}$.

The transmission rates, for the same film, for oxygen, carbon dioxide and water vapour should be as follows:

oxygen:-	a transmission rate of greater than 1.2×10^{-17}
carbon dioxide:-	a transmission rate of greater than 4×10^{-17}
water vapour:-	a transmission rate of less than 20×10^{-16}.

With thin packages made from a polyethylene film having approximately the minimum values of these permeabilities to oxygen and carbon dioxide, the area of polyethylene surface exposed to the air, per gram of the combination of J3 nematodes with an attapulgite clay carrier, for storage at 23°C, should be at least 2 cm^2.

When transporting the wrapped nematodes and their carrier medium, two convenient materials for supporting the packages in the container are corrugated cardboard and an open structure nylon scourer pad material (which has a mesh structure made from nylon thread). However, any suitable material may be used to support (and in many cases, to separate) the packages. Preferably the container into which the packages are placed for transportation is an

insulating container (for example, a container made from a foamed polystyrene material). When such an insulating container is used, a block of a heat absorbing material, to maintain the temperature within the container at a reduced value, but not below 4°C, will normally be included within the container.

The way in which the nematodes and certain carrier media are formed into a combination to initiate anhydrobiosis prior to being wrapped as thin packages is also important for some species of nematodes.

The present invention also encompasses third stage juvenile (J3) entomopathogenic nematodes which have been wrapped in a polymer film in accordance with the present invention, and (for transportation of the nematodes) a container of such packaged nematodes.

These and other features of the present invention will be discussed further in the following description of implementations of the invention, which is provided by way of example only. In the following description, reference will be made to the accompanying drawing.

Brief Description of the Drawing

Figure 1 is a schematic section through a container within which several layers of thin packages of nematodes and their associated carrier medium have been placed, in layers, in accordance with the present invention.

Detailed Description of Implementations of the Present Invention

The first step of the present invention is the initiation or induction of anhydrobiosis to reduce the oxygen consumption rate of J3 entomopathogenic nematodes. The J3 nematodes and their associated carrier medium are then effectively wrapped in a polymer film in a manner such that (i) oxygen can reach all of the nematodes in the package, and (ii) the water activity of the nematodes and carrier combination is maintained at a suitable level.

Polyethylene film which is permeable to oxygen and carbon dioxide, but relatively impermeable to water vapour, has been found to be a suitable packaging material. The permeability to oxygen is the most important feature. A polymer film having a relatively high permeability to water vapour is not barred from use in the present invention, for (as will be shown later in this specification) it is possible to ensure that the air surrounding a package has a high relative humidity, so that the moisture content of the package, and hence the water activity of the nematode carrier medium, will not be reduced by transfer of water vapour out of the package.

The thicker the polyethylene (or other polymer material) film, the lower its permeability to oxygen. The film used in the present invention should have a thickness in the range of from 10μ (microns) to 150μ. In the case of a polyethylene film having a thickness of 50μ, the permeabilities and transport data given above are relevant. The initial testing of the present invention by the

The initial testing of the present invention by the inventors was carried out using polyethylene films having permeabilities to oxygen in the range of from 1.8×10^{-16} to 27×10^{-16} mole.$m^{-1}s^{-1}pa^{-1}$.

One film which has been used very successfully for the packaging of J3 nematodes in accordance with the present invention is a polyethylene film containing an adsorbent, of the type described in the specification of US patent No 4,847,145 (to Matsuo Matsui). Such a film is sold in Australia, made into bags, under the trade marks **EVERFRESH** and **ROB'S**. Bags, in fact, are very convenient for use in the present invention, but there is no reason why a suitable package cannot be made from a polyethylene film having the capability of forming a weak seal when overlapped with itself, possibly with the assistance of an adhesive strip or tape.

Controlled permeability polymer films, of which the polymer material is a polyolefin (for example, polyethylene or polypropylene), a polyester (for example, polyethylene terephthalate or polybutylene terephthalate), a vinyl polymer (such as polyvinyl chloride, polyvinyl acetate, ethylene-vinyl acetate copolymers and ethylene-vinyl alcohol copolymers), a polycarbonate, a polystyrene polyalkylene oxide polymer (including polyethylene oxide polymer), or blends of such materials, and including an inert filler selected from a range of materials, but preferably an inorganic filler selected from alumina, silica, pumice and derivatives thereof, are described in the specification of International patent application

No PCT/AU91/00246. Such films may be used in the present invention.

Other polymer films that may be used as the wrapping material of the present invention include:

(a) the film marketed by Du Pont de Nemours and Co. under the trade mark TYVEK;

(b) the perforated polymeric film which is described in the specification of European Patent Office Publication No 351,116;

(c) the perforated film produced by Mitsubishi Gas Chemical Company, and described in the specification of Japanese patent application No 04-210,552 (1992);

(d) the three layer film produced by Mitsui Toatsu Chemicals and described in European Patent Publication No 356,161;

(e) the polyethylene film described in the specification of International patent application No PCT/AU90/00400; and

(f) the microporous film produced by Hercules, Inc and described in the specification of Australian patent application No 79395/87.

This list is not exhaustive.

The use of attapulgite clay as a carrier medium for J3 entomopathogenic nematodes was developed by one of the present inventors. As there has been significant testing of the present invention using nematodes combined with this carrier medium, implementations of the invention using this carrier medium will now be discussed.

To form a package containing third stage juvenile nematodes of the Heterorhabditis species, the nematodes may be mixed with finely ground attapulgite clay, to form an homogeneous mixture that can be thickly spread within a polyethylene bag (or on a layer of polyethylene film) and formed into a thin package of the mixture of nematodes and carrier medium after the required exposure to air for the induction of anhydrobiosis.

Third stage juveniles of all entomopathogenic nematode species still have the J2 sheath or cuticle (a membrane) from their previous stage as a protective layer. In the Steinernema species, this J2 sheath is easily destroyed by abrasion when the J3 nematodes are mixed with ground attapulgite clay. This removal of the J2 sheath causes the bodies of the nematodes to be abraded by the clay particles, which results in the death of the nematodes. Thus a different technique from that described above for the Heterorhabditis species nematodes has to be used when making packages of J3 nematodes of the Steinernema species (this different technique can also be used, of course, to form packages of J3 nematodes of the Heterorhabditis species).

To form a package of J3 nematodes of the Steinernema species, a thin layer of an aqueous cream of the nematodes, having a viscosity such that a water activity of 97 per cent is obtained when this aqueous cream is combined with a known quantity of ground attapulgite clay, is spread on a sheet of polyethylene film (or other wrapping medium). Half of the known quantity of finely ground attapulgite

clay is then applied to one side of the layer of nematodes, to form a layer of the carrier medium on top of the layer of nematodes. Using another part of the sheet of polyethylene film (usually the other side sheet of an envelope or bag made from the polyethylene film), the layers of carrier medium and nematodes are inverted, so that the carrier medium layer is supported on polyethylene film and the nematode layer is uppermost and exposed. The other half of the selected quantity of finely ground attapulgite clay is then applied to the other exposed surface of the nematode layer, to form a thin sandwich of nematode cream between two layers of carrier medium.

An alternative way of producing such a thin sandwich of a layer of a cream of nematodes between two layers of carrier medium is to form a first layer comprising one half of the required quantity of finely ground attapulgite clay on a sheet of polyethylene (or other material) film, spread the nematode cream on this layer, then apply the remainder of the required quantity of ground attapulgite clay as a layer on top of the layer of the cream of nematodes.

It will be appreciated by those familiar with the use of entomopathogenic nematodes that when the nematodes and attapulgite clay come into contact, and are mixed together, anhydrobiosis begins, although the equilibrium state takes some time to achieve. The consequence of this start of the "drying-out" of the nematodes is that the nematodes use less oxygen than when they are in their active state. Accordingly, the mixture of nematodes and attapulgite clay is left for at least 15 hours, and preferably for a period

of 1 or 2 days (although there is no rigid upper limit to this time period), at a warm temperature, until the respiration rate of the nematodes is reduced, before sealing the polyethylene film around the mixture of nematodes and carrier medium, or to form a thin package. However, as noted above, if polymer films having a very high permeability to oxygen (which are currently very expensive) are available to wrap the J3 nematodes and their carrier medium, it may not be necessary to induce anhydrobiosis before wrapping the nematodes in the polymer film.

When forming such samples of J3 nematodes and their associated carrier medium (attapulgite clay) into thin packages, particles of polyacrylamide gel, or of another highly water absorbent material, having a water content sufficient to maintain a relative humidity of from 85 per cent to 99 per cent, may be included within the envelope of the polymer film.

If the carrier medium for the nematodes is not attapulgite clay, but is the currently preferred carrier medium, polyacrylamide gel or one of the other highly water absorbent materials (such as methyl cellulose or polyacrylate starch gel powder) which are described in the specification of International patent application No PCT/AU93/00465, then there is no abrasion of the J2 sheath or cuticle when J3 nematodes of the Steinernema species are mixed with the partially hydrated carrier medium. Thus the layering technique for producing a sandwich of the nematode cream between two layers of the

carrier medium, described above, does not have to be used with the preferred carrier media.

Figure 1 shows, schematically, a series of thin packages 10 containing J3 entomopathogenic nematodes and their associated carrier medium, packed in accordance with the present invention and placed within an insulating container 11 for transportation. The container 11, which has a lid 12, is preferably made from foamed polystyrene. Such a container is light-weight, is quite strong, and has good insulating properties.

The thin packages of nematodes and carrier material are in layers. Each layer is supported on a support 13 of a material which allows air within the container to come into contact with each of the large faces of the thin packages 10. Corrugated cardboard sheets, which are depicted schematically in Figure 1, have been found to be very suitable as the supports 13. Sheets of open-mesh nylon scourer pad material, about 7 mm thick, have also been found to be eminently suitable for use as the supports 13. However, any other suitable material may be used for the supports 13. As noted above, the normal requirement is that the supports 13 enable the packages 10 to be placed in the container 11 in a manner such that at least 2 cm^2 of the polyethylene material is exposed per gram of the combination of nematodes and carrier medium in the package.

The packed container illustrated in Figure 1 also carries (i) a "cold pack" 14 and (ii) a humidifying pack 15. Such packs 14 and 15 will not be required in all circumstances.

The heat absorbing cold pack 14 preferably comprises a frozen gel composition within an insulated container, so constructed that the cold pack maintains the temperature within the container 11 at a low value, which does not fall below 4°C, for a considerable time. The third stage juvenile nematodes have a reduced respiration rate at lower temperatures, so clearly it is advantageous (though not essential) to keep the stored nematodes at a temperature which is as low as practicable, but not below 4°C.

The humidifying pack 15 is preferably a porous container (for example, a fabric mesh bag) containing polyacrylamide gel particles, or particles of another highly water absorbent material, hydrated to the extent that they maintain a relative humidity in the container at a value of from 85 per cent to 100 per cent, preferably from 95 per cent to 99 per cent, and ideally at a value corresponding to the optimal water activity for the nematodes that have been packaged (which, as noted above, is about 97 per cent for the Steinernema carpocapsae species of nematode).

In practice, it will usually be most convenient to maintain the relative humidity within the container at about 100 per cent, using hydrated polyacrylamide gel particles as the humidifying agent. Polyacrylamide gel particles can absorb up to about 300 times their dry weight of water without becoming a liquid. The hydrated gel acts as a reservior of water which maintains a relative humidity of, effectively, 100 per cent until its water content has dropped to a value of less than 6 times the dry weight of the polyacrylamide gel. Maintaining a relative humidity of 100 per cent in the container stops the drying out of the packaged J3 nematodes and their carrier medium, and because there is

only a small difference between the relative humidity within the packages and the relative humidity of the air in the container, there is little water transport through the polymer film used to package the J3 nematodes.

The arrangement illustrated in Figure 1 (but without the packs 14 and 15) has been used to store nematodes at 23°C for up to six months, without any loss of activity of the nematodes when they are removed from the carrier medium by conventional methods. It is believed that, using a humidifying pack 15, the storage time at 23°C should be extendible to one year or even longer.

Another benefit possessed by entomopathogenic nematodes that have been wrapped and stored in accordance with the present invention is that fungal infection of the nematodes is rare during storage, whereas (as noted earlier in this specification) it is regularly experienced with entomopathogenic J3 nematodes stored in the conventional manner - that is, in a container with a lid, with apertures in the lid or around the top edge(s) of the container wall(s). This benefit will also be experienced when J3 nematodes are wrapped in larger packages using a polymer film having a high permeability to oxygen, and when they are packaged in a shallow open box which is closed by a polymer film having a very high permeability to oxygen.

Yet another benefit of the arrangement of Figure 1 is the reduction in the volume of the container that is required to transport a given quantity of nematodes, thus making it easier to transport the nematodes by air freight.

Users of stored nematodes will wish to ensure that they are distributing active nematodes. When using the present invention, periodic weighing of the package containing the J3 nematodes and their carrier medium can be used to test whether the package has been maintained at an acceptable humidity level. If the package loses more than 10 per cent of its weight, steps should be taken to ensure that the water activity in the package does not fall to a level which is too low to permit the successful revival of the stored nematodes.

Those skilled in this art will appreciate that the examples of the present invention described above merely illustrate aspects of the present invention, and modification of those examples is possible, without departing from the present inventive concept, to provide packages of nematodes tailored to reflect (a) the number of nematodes in the package, which affects the oxygen requirements, (b) the time for which the package is expected to be stored, and (c) the relative humidity in the storage environment.

CLAIMS

1. A method of packaging infective third stage juvenile (J3) entomopathogenic nematodes for transportation and/or storage, said method comprising the steps of:
   (a) combining an aqueous cream of the nematodes and a carrier medium in proportions which provide a water activity for the combination in the range of from 0.85 to 0.99;
   (b) exposing the combination obtained by step (a) to air for a period sufficient to induce anhydrobiosis of the nematodes; and
   (c) forming at least one package of the combination of nematodes and carrier medium, by surrounding a quantity of the combination of nematodes and carrier medium with a film of a polymer material having a permeability to oxygen;

2. A method as defined in claim 1, in which the period of exposure to induce anhydrobiosis of the nematodes is at least 15 hours.

3. A method as defined in claim 1 or claim 2, in which said film of a polymer material is a film having a thickness of from 10μ to 150μ and a permeability to oxygen which is greater than 1.8×10^{-16} mole.$m^{-1}s^{-1}pa^{-1}$.

4. A method as defined in any preceding claim, in which said film of a polymer material has a thickness of about 50µ, and has:
   (a) a permeability to oxygen which is greater than 6×10^{-16} mole.$m^{-1}s^{-1}pa^{-1}$;

(b) a permeability to carbon dioxide which is greater than 20×10^{-16} $mole.m^{-1}s^{-1}pa^{-1}$;

(c) a permeability to water vapour which is less than 1000×10^{-16} $mole.m^{-1}s^{-1}pa^{-1}$;

(d) an oxygen transmission rate which is greater than 1.2×10^{-1};

(e) a carbon dioxide transmission rate which is greater than 4×10^{-17}; and

(f) a water vapour transmission rate which is less than 20×10^{-16}.

5. A method as defined in any preceding claim, in which said film of a polymer material is a polyethylene film.

6. A method as defined in any one of claims 1 to 4, in which said film of a plastic material is a film of a material selected from the group consisting of:-

(a) polyethylene containing an absorbent material;

(b) a polyolefin containing an inorganic filler;

(c) a polyester containing an inorganic filler;

(d) a vinyl polymer containing an inorganic filler;

(e) a polycarbonate containing an inorganic filler;

(f) a polystyrene containing an inorganic filler;

(g) a mixture of the polymers recited in (b) to (f) of this claim, containing an inorganic filler; and

(h) any other plastic material recited in the description of the present invention.

7. A method as defined in any one of claims 3, 4, 5 and 6, in which the polymer film has a relatively low

permeability, and said or each package is a thin package.

8. A method of packaging infective third stage juvenile (J3) entomopathogenic nematodes for transportation and/or storage, said method comprising the steps of:
(a) combining an aqueous cream of the nematodes and a carrier medium in proportions which provide a water activity for the combination in the range of from 0.85 to 0.99; and
(c) forming at least one package of the combination of nematodes and carrier medium, by surrounding a quantity of the combination of nematodes and carrier medium with a film of a polymer material having a very high permeability to oxygen.

9. A method of packaging infective third stage juvenile (J3) entomopathogenic nematodes for transportation and/or storage, said method comprising the steps of:
(a) combining an aqueous cream of the nematodes and a carrier medium in proportions which provide a water activity for the combination in the range of from 0.85 to 0.99; and
(b) placing at least one sample of the combination of nematodes and carrier material into an open box and closing said box with a membrane comprising a film of polymer material which is highly permeable to oxygen.

10. A method as defined in any preceding claim, in which said carrier medium is a partially hydrated highly water-absorbent material.

11. A method as defined in claim 10, in which said carrier medium is polyacrylamide gel.

12. A method as defined in claim 10, in which said carrier medium is selected from the group consisting of methyl cellulose powder, polyacrylate starch gel, and starch powder.

13. A method as defined in any one of claims 1 to 9, in which said carrier material is attapulgite clay.

14. A method as defined in claim 13, in which the third stage juvenile nematodes are nematodes of the Steinernema species, and said combination of nematodes and carrier medium comprises a layer of the aqueous cream of nematodes between two layers of the attapulgite clay.

15. A method as defined in claim 13, in which the third stage juvenile nematodes are nematodes of the Heterorhabditis species and said combination of nematodes and carrier medium comprises an intimate mixture of the aqueous cream of nematodes and the attapulgite clay.

16. A method as defined in claim 13, in which the third stage juvenile nematodes are nematodes of the Heterorhabditis species and said combination of nematodes and carrier medium comprises a layer of the aqueous cream of nematodes between two layers of the attapulgite clay.

17. A package containing a combination of third stage juvenile entomopathogenic nematodes and a carrier medium, produced by the method of any preceding claim.

18. A method as defined in any one of claims 1 to 16, including the additional steps of
(1) supporting said or each package within a container on at least one layer of a supporting material which allows air to contact a substantial proportion of the outer surface area of said or each package; and
(2) maintaining the relative humidity within said container at a value in the range of from 85 per cent to 100 per cent.

19. A method as defined in claim 18, in which the relative humidity within said container is maintained at a value in the range of from 95 per cent to 100 per cent.

20. A method as defined in claim 18, in which the relative humidity within said container is maintained at a value which corresponds to the optimal water activity of the J3 nematodes.

21. A method as defined in any one of claims 18 to 20, in which said supporting material supports said or each package in a manner such that at least 2 cm^2 of said film is exposed per gram of said combination of nematodes and carrier material.

22. A method as defined in any one of claims 18 to 21, in which said supporting material comprises corrugated

cardboard or an open mesh material formed from nylon threads.

23. A method as defined in any one of claims 18 to 22, in which said package or at least one of said packages contains a quantity of polyacrylamide gel which is hydrated to the extent required to maintain a relative humidity within its associated package of about 97 per cent.

24. A method as defined in any one of claims 18 to 22, in which said container also contains a humidifying pack.

25. A method as defined in claim 24, in which said humidifying pack comprises a porous enclosure which contains particles of a highly water-absorbent material which is hydrated to the extent required to maintain a relative humidity within said container
(a) in the range of from 85 per cent to 100 per cent;
(b) in the range of from 89 per cent to 100 per cent; or
(c) of about 97 per cent.

26. A method as defined in claim 25, in which the highly water-absorbent material in said humidifying pack is polyacrylamide gel.

27. A method as defined in any one of claims 18 to 26, in which said container is made of an insulating material and the interior of said container is maintained at a low temperature which is not less than 4°C.

28. A method as defined in claim 27, in which said container also contains a cooling pack comprising a container of a frozen gel composition.

29. A container of at least one package of third stage juvenile entomopathogenic nematodes in combination with a carrier material, produced by the method of any one of claims 18 to 28.

30. A method of packaging infective third stage juvenile entomopathogenic nematodes for transportation and/or storage as defined in claim 1, substantially as hereinbefore described.

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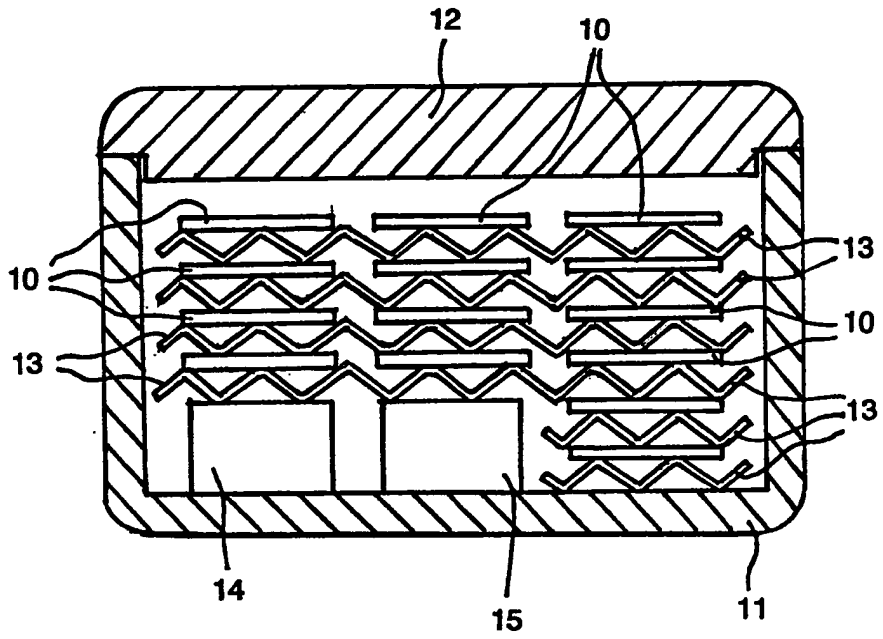

FIG.1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 94/00102

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[5] A01N 1/00, 63/00, B65D 81/18, 85/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[5] A01N 1/00, 63/00, B65D 81/18, 85/50

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

DERWENT, JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
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Y	WO,A1,89/07446 (BIOSYS) 24 August 1989 (24.08.89) whole document	1-30
X	EP,A2,0256873 (BIOSIS PARTNERS) 24 February 1988 (24.02.88) whole document	1-30
Y	WO,A1,90/10063 (BIOSYS) 7 September 1990 (07.09.90) whole document	1-30

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 June 1994 (09.06.94)	29 June 1994 (29.06.94)

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	US,A,4615883 (NELSEN ET AL) 7 October 1986 (07.10.86) whole document	1-30
A	AU,A,33839/93 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 2 September 1993 (02.09.93) whole document	1-30
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END OF ANNEXE